POPULATION STATUS AND CONSERVATION PRIORITY OF *Zygodon leptobolax,* AN ENDEMIC MOSS OF SOUTH AFRICA

N.M. WHEAT

BOTANY HONOURS 2004

PLANT CONSERVATION MODULE

SUPERVISOR: T.A. HEDDERSON

POPULATION STATUS AND CONSERVATION PRIORITY OF *Zygodon leptobolax*, AN ENDEMIC MOSS OF SOUTH AFRICA

N.M. Wheat

ABSTRACT

Zygodon leptobolax is endemic to the eastern slopes of Table Mountain, South Africa, where it grows on the bark of trees. The original host species is unknown and all recent populations have been found on introduced *Quercus* species. The plans are in place to remove alien trees from Table Mountain. As host trees are removed, so too will populations of *Z. leptobolax*. This could lead to the extinction of the moss within the next few years. This study examined populations and determined their conservation priority, via a population census combined with molecular analysis. 43 trees were found to be hosting populations, all hosts being *Quercus* species, older than 100 years, and mostly with corrugated bark. The majority of populations were considered to be small, and only four had sporophytes. Molecular analysis of the *psb*A region showed *Z. leptobolax* to be genetically distinct from other similar species. In addition, *Z. leptobolax* can be considered rare and is currently threatened. Hence, this moss should be afforded the highest conservation priority and a conservation plan should be devised to protect it

INTRODUCTION

The genus *Zygodon* consists of about 90 species, which are mostly found in temperate and tropical regions of the southern hemisphere. Several species occur in South Africa, where they grow on the bark of trees or on soil in montane forests (Magill & van Rooy 1998). *Zygodon leptobolax* is endemic to the eastern slopes of Table Mountain, in the Western Cape region of South Africa. It is almost identical to *Zygodon intermedius*, the only morphological difference between the two being the

grouping of sexual organs on the gametophyte. In addition, these two mosses have disjunctive distributions, with *Z. intermedius* being found in the Eastern Cape of South Africa as well as in Asia and tropical America, while *Z. leptobolax* is restricted to Table Mountain.

Z. leptobolax is known to grow on the bark of forest trees, where it can occur as single shoots or in larger aggregations. The host species of the original collection is unknown and recent collections have all been found on the bark of large specimens of *Quercus* species. *Quercus* species are not indigenous to South Africa and were introduced to the region in the 19th century. It appears that *Z. leptobolax* grows on large individuals of *Quercus* species, due to similarities between them and the original indigenous host species. It seems likely that potential indigenous hosts have not yet reached sufficient size to sustain populations of *Z. leptobolax*.

South Africa is one of the world leaders in the control of alien invasive plants. After several studies showed that invasive alien trees growing in mountain water catchment areas reduce streamflow, aggressive removal campaigns were initiated (Le Maitre et. al. 2002). The removal efforts focused on a few specific invasive species e.g. Acacia and pine spp. *Quercus* species were not included in the invasive alien removal plan because, although they are an alien species, they aren't invasive. However, recent plans have been initiated to remove all alien plants from Table Mountain, not just the invasive species. This may pose a serious conservation threat to *Z. leptobolax*, since it has only been known to grow on *Quercus* species. As host trees are removed, so too will core populations of *Z. leptobolax*. In the absence of new colonisation sites or an alternative conservation method, the species is likely to become extinct in the next few years.

In this paper, the conservation priority of *Z. leptobolax* will be determined, by means of a population census and examination of molecular data, in an attempt to address the key question: Should conservation efforts be directed towards protecting *Zygodon leptobolax*?

METHODS

Study plant

This study focused on populations of the moss *Zygodon leptobolax* C. Muller (Orthotrichales: Orthotrichaceae). Stems, averaging 0.5cm in length, bear small oblong-lanceolate leaves. Leaves are costate and have many papillae along their margins. *Z. leptobolax* is synoicous, and therefore monoecious, and has both antheridia and archegonia in clusters together. Seta are roughly 4.5cm or longer, each bearing a brown capsule. When ripe, small spores are discharged through an operculum (Sim 1926).

Population census

This study was performed on populations of *Zygodon leptobolax* growing on trees on the eastern slopes of Table Mountain. An area extending from Rhodes Memorial (33° 46.78S, 018° 57.26E) to Kirstenbosch National Botanical Gardens (33° 59.17S, 18° 25.99E) was examined, and included all forested areas above the main highway (M3).

The study area was divided into sections of roughly similar size, and trees in each section (both alien and indigenous) were examined for the presence of *Z. leptobolax*. Once a population was found, the host tree was identified and the coordinates for the location were recorded. Additional details of the bark texture, location of the population on the trunk and size of the population were recorded. Bark texture was divided into four categories, large corrugations, intermediate sized corrugations, mild corrugations and smooth bark. Population size was also categorically recorded, with size being divided into 4 classes, ranging from single shoots to extensive populations that covered large areas of the tree trunk. Several small specimens were collected from different sites, placed in paper collection envelopes, and left to air-dry upon return to the laboratory. These were later used in DNA analysis.

A map showing the geographical distribution of trees hosting populations of *Z. leptobolax* was constructed using Geographical Information Systems (GIS) software (ArcView v3.2, Environmental Systems Research Institute, Inc.).

Molecular analysis

DNA extraction, amplification and sequencing

Molecular analysis was performed on four herbarium samples, *Z. intermedius*, *Z. baumgartneri*, *Z. runcinatus* and *Z. viridissimus*, as well as five fresh samples of *Z. leptobolax* that were collected during the population census. Approximately 0.5ml of each dried specimen was placed in a 1.5ml micro-centrifuge tube for extraction. DNA was extracted following a modified version of the protocol outlined by Gawel and Jarret (1991). Each sample was ground in a mortar and pestle with 700µl of hexadectyltrimethylammonium (CTAB) and 1µl of β-mercaptoethanol. Once ground, the samples were returned to the 1.5ml micro-centrifuge tubes and heated in a water bath at 65°C for approximately 30 minutes. 600µl of chloroform-isoamyl alcohol (24:1 v/v) was added to each sample and mixed by inversion. Samples were spun in a micro-centrifuge at 12000 rpm for 5 minutes, after which the supernatant was transferred to clean 1.5ml micro-centrifuge tubes. An equal volume of ice-cold isopropanol was added and mixed briefly by inversion. Samples were placed in the fridge for 1 hour to precipitate the DNA.

Chilled samples were spun at 12000 rpm for 5 minutes to recover DNA. The resulting DNA pellets were washed with 250µl of 75% ethanol, which was discarded, and left to air dry. DNA was re-suspended in 100µl of autoclaved double-distilled water (PCR water) and stored in the fridge. Dilutions of the raw DNA solution were made by adding 45µl of DNA solution to 5µl of PCR water. Dilutions and raw DNA extract were stored in the fridge.

The primers *psb*A and *trn*H (Hamilton 1999) were used to isolate the plastid *psb*A region. The target region was amplified by the Polymerase Chain Reaction (PCR). 3µl of each DNA template was placed in a micro-centrifuge tube with 27µl of master mix, containing 14.65µl PCR water, 3µl 10x NH_4 buffer, 6µl 25mM $MgCl_2$, 1.2µl dNTP, 1µl of each primer and 0.15µ SUPERTHERM™ (Bioline) DNA polymerase. Thermo-cycling consisted of the following conditions: initial denaturation at 94°C for 2 minutes, followed by 30 cycles of 94°C for 1 minute, 52°C for 1 minute and 72°C for 2 minutes. After 30 cycles, there was a final polymerisation step at 72°C for 7 minutes.

Amplified DNA was cleaned using a GFX™ PCR DNA and Gel Band Purification Kit (Amersham Biosciences). Each GFX column consisted of a glass fibre matrix and a collection tube. 500µl of capture buffer, consisting of a buffered solution containing acetate and chaotrope, was placed in each column to which the amplified DNA solution was added. The columns were spun at 14000 rpm for 30 seconds, and the resulting flow-through was discarded. The glass fibre matrix was then washed with 500µl of wash buffer, consisting 10mM Tris-HCl and 1mM EDTA diluted to 80% with 100% ethanol. 30µl of PCR water was applied directly to the top of the glass fibre matrix, and the columns were left to stand at room temperature for 5 minutes. They were then spun at 14000 rpm for 1 minute to recover the purified DNA.

Cycle sequencing was done on the cleaned amplified DNA by PCR in 10µl volumes. Each 10µl sample contained 1µl to 4µl of DNA template, 2µl BigDye® Terminator v3.1 Cycle Sequencing TRR (Applied Biosystems), 1µl 10x NH_4 buffer, 0.16µl primer and the remaining volume of PCR water. Cycle sequencing products were resolved on an ABI PRISM 3100 Genetic Analyser for direct nucleotide sequencing.

Phylogenetic analysis

Sequences were assembled using SeqMan (LaserGene System Software, DNAStar, Inc.). Sequence ends were trimmed and aligned automatically using MegAlign (LaserGene System Software, DNAStar, Inc.). Sequence data were analysed using PAUP (v4.06 for Macintosh). A midpoint rooted phylogenetic tree was constructed and a pairwise difference matrix generated.

Propagule viability

Experiments were to be conducted on spores to determine their germinability. However, mature sporophytes were unavailable at the time of the study. Sporophytes were observed on populations *in situ*, but the capsules were either immature or had already dispersed their spores. Hence, this aspect of the study could not be performed. Study of the reproductive capacity of *Z. leptobolax* was reduced to recording the locations of populations bearing sporophytes.

RESULTS

Population census

A total of 43 trees were found to be hosting populations of Zygodon leptobolax, which were arranged into three groups (Fig. 2). All host trees were Quercus species aged between 100 and 150 years (Dr. E.C. February, personal communication). Although all trees were roughly the same age, diameter at breast height (dbh) varied among them, ranging between 117cm and 398cm. Trees were evenly distributed among size classes within this range (Fig. 1). However, not all potential host trees within this size range harboured populations of *Z. leptobolax*. This indicates that even though *Z. leptobolax* only grows on old and established trees, the actual size of the trunk is no indication as to whether a tree is a suitable host.

The majority of host trees (88.4%) had bark with high to intermediate levels of corrugation (Fig.3). This indicates that *Z. leptobolax* tends to grow on corrugated, as opposed to smooth, bark. This supports the previous result, in that as *Quercus* species age, their bark becomes more corrugated. Hence, older trees with more corrugated bark are more likely to host populations of the moss.

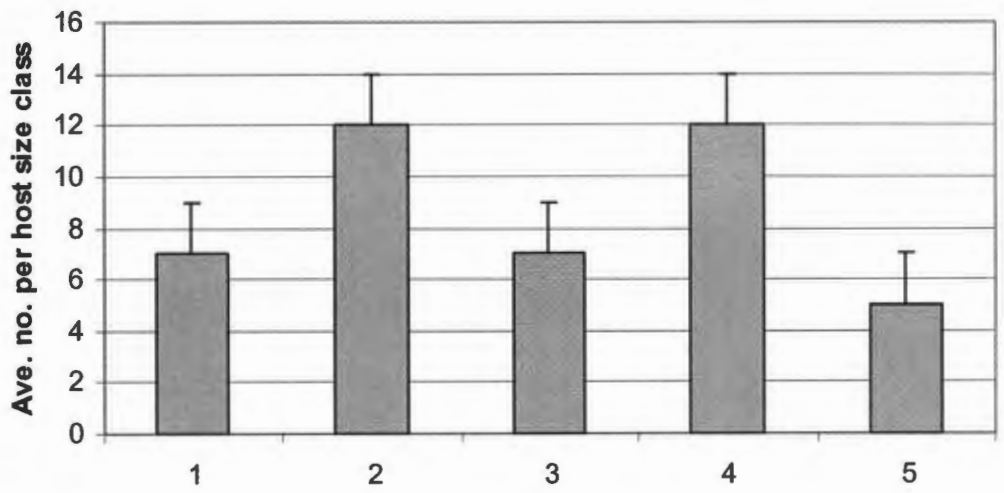

Fig. 1: Mean number of host trees per size class, measured as diameter at breast height in cm, class 1 = 116.95–148.45, class 2 =148.46–179.65, 3 = 179.66–210.85, 4 = 210.86–242.05, 5

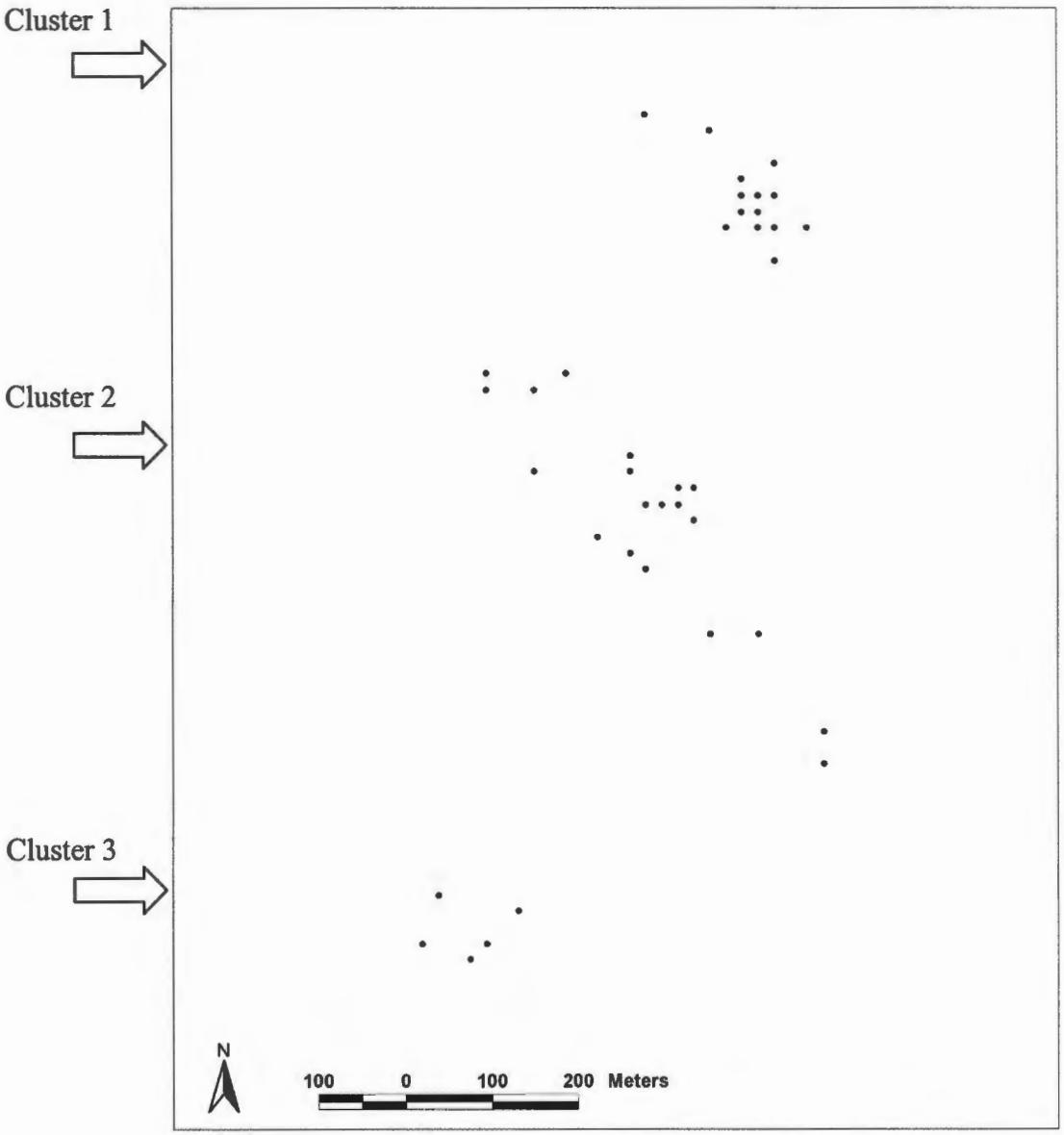

Fig. 2: Map showing the geographical distribution of trees on the eastern slopes of Table Mountain hosting populations of *Zygodon leptobolax*. Cluster 1 represents populations found in First Waterfall Ravine on Devil's Peak. Cluster 2 represents populations found in Duiwekloof Ravine, also on Devil's Peak. Cluster 3 represents those populations found in the vicinity of Second Watefall Ravine, also Devil's Peak. All three clusters were on similar soil, near to seasonal riverbeds. In addition, nearly all populations in cluster 1 were found in association with *Hypnum* spp.

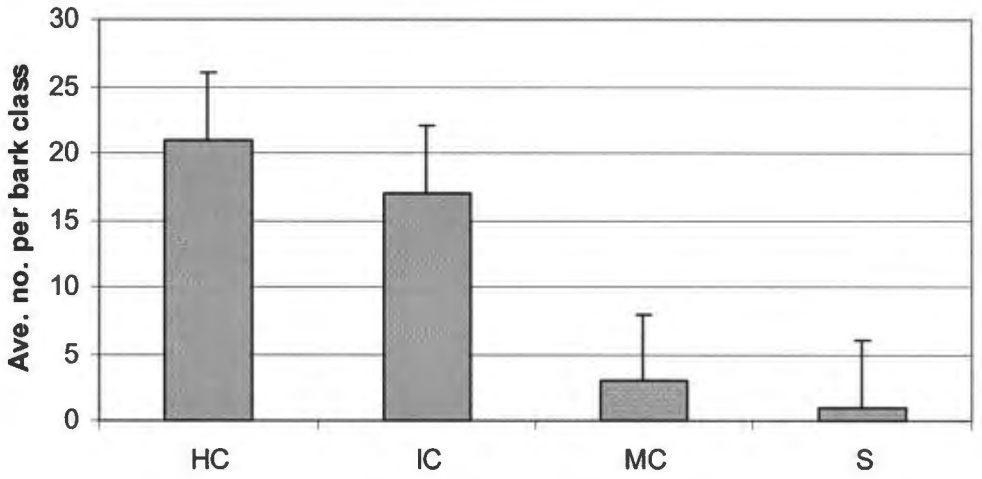

Fig. 3: Mean number of host trees per bark category, where HC = highly corrugated bark, IC = intermediately corrugated bark, MC = mildly corrugated bark and S = smooth bark

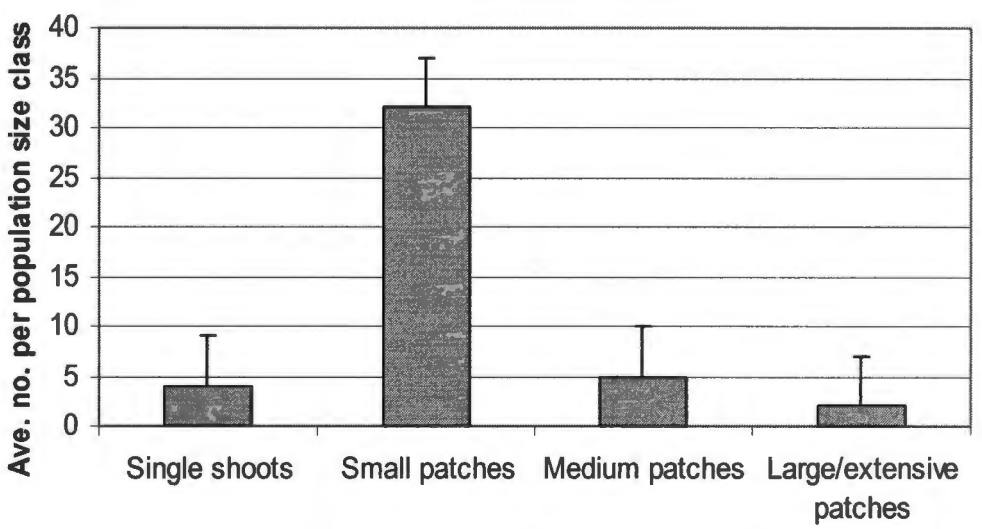

Fig. 4: Mean number of populations per size class. Small patches consisted of fewer than 20 shoots, medium patches of between 20 and 50 shoots and large patches consisted of more than 50 shoots.

The majority of populations (74.4%) were small in size, consisting of fewer than 20 shoots each (Fig. 4). Only two populations were considered to be large or extensive i.e. consisting of more than 50 shoots. This indicates that *Z. leptobolax* is not readily reproducing either sexually, via spores, or asexually, via gemmae, and thus populations remains small in most instances. In addition, only four populations showed signs of sexual reproduction, in the form of sporophytes. In the populations where sporophytes had formed, they were numerous. It was not clear whether sporophytes were the product of self-fertilisation. However, if self-fertilisation was usually practised, a higher number of populations could be expected to bear sporophytes.

Molecular analysis

Molecular analysis of the plastid gene region *psb*A resulted in a phylogenetic tree incorporating five species of *Zygodon* (Fig. 5). This showed that there was no within species genetic variation for the fresh samples of *Z. leptobolax* collected from the study area The most morphologically similar species to *Z. leptobolax*, *Z. intermedius*, was shown to have considerable genetic difference from the study species (Table 1). In addition, the species most genetically similar to *Z. leptobolax* was shown to be *Z. runcinatus,* which is the most dissimilar morphologically to *Z. leptobolax*.

Table 1: Pairwise distance matrix showing the amount of difference among the *Zygodon* species analysed

		1	2	3	4	5	6	7	8
1	Z. intermedius								
2	Z. baumgertneri	0.01036							
3	Z. runcinatus	0.02618	0.02618						
4	Z. viridissimus	0.01554	0.00518	0.03136					
5	Z. leptobolax pop. 1	0.02086	0.02086	0.02094	0.02604				
6	Z. leptobolax pop. 2	0.02086	0.02086	0.02094	0.02604	0.00000			
7	Z. leptobolax pop. 3	0.02086	0.02086	0.02094	0.02604	0.00000	0.00000		
8	Z. leptobolax pop. 4	0.02086	0.02086	0.02094	0.02604	0.00000	0.00000	0.00000	
9	Z. leptobolax pop. 5	0.02086	0.02086	0.02094	0.02604	0.00000	0.00000	0.00000	0.00000

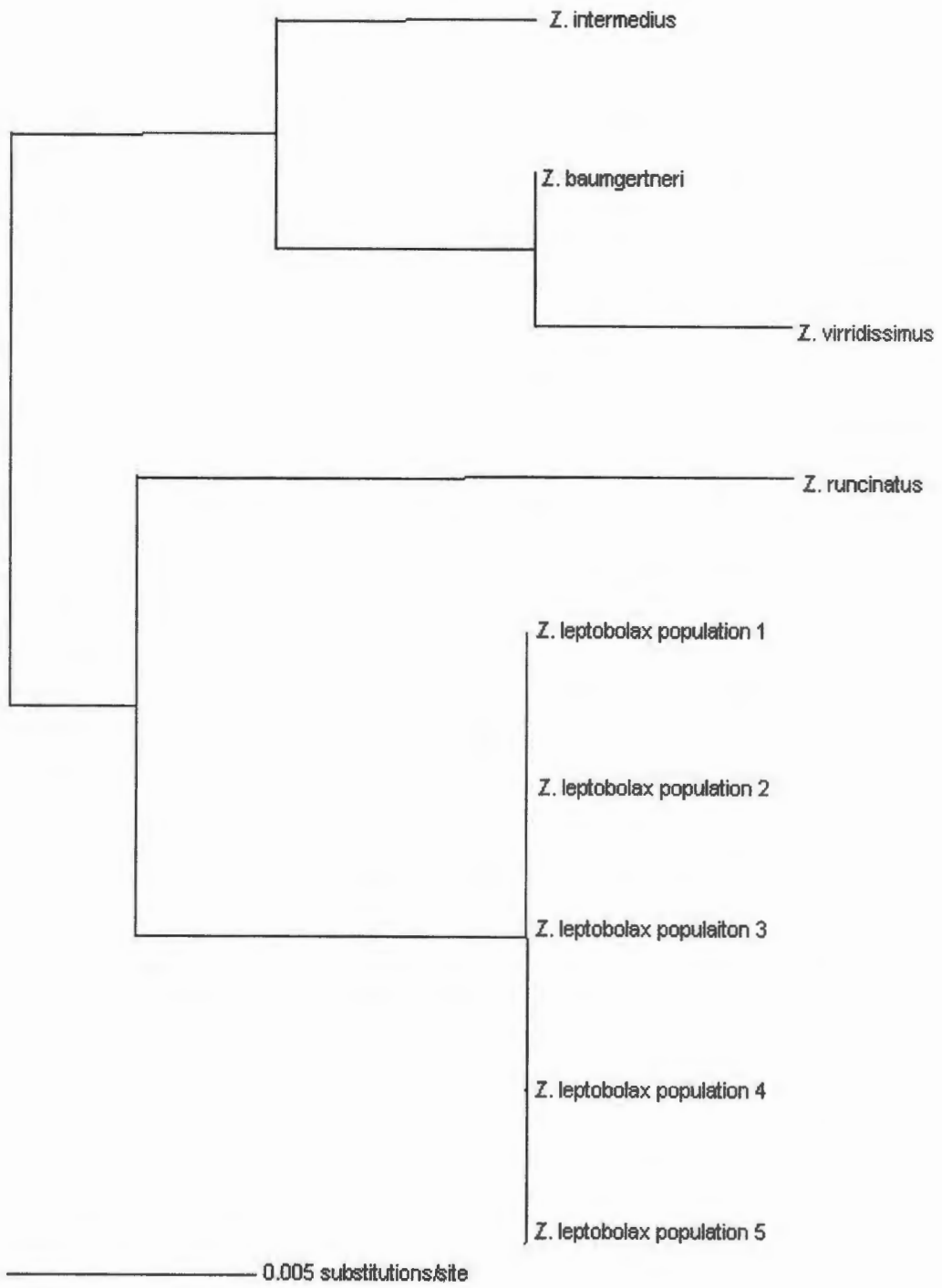

Fig. 5: The midpoint rooted phylogenetic tree based on the plastid gene region *psb*A.

DISCUSSION

This study examined the population status of *Zygodon leptobolax* and addressed two main questions: 1) What is the conservation priority of this species? and 2) Should conservation efforts be directed toward protection of *Z. leptobolax*?

Z. leptobolax is unknown from indigenous trees, but is found to grow on *Quercus* trees over 100 years old, mainly with rough bark. Trees younger than 100 years appear to be unsuitable hosts. This may be due to the nature of the bark of *Quercus* trees, rather than a direct result of their age. As they age, their bark usually gets more corrugated. Large grooves form, running vertically up the trunk. These grooves may offer shelter to *Z. leptobolax*, allowing spores to settle, germinate and grow in a more protected environment than could be offered by smoother bark. In addition to this protective function, the corrugated bark could channel water down the length of the trunk. For a moss, which relies on external water, this may be a way of ensuring lasting moisture when rain is intermittent. The crevices in the grooves may stay wet for longer than smooth bark could, resulting in a secure, moist environment, which would be ideal for a moss to grow in. For this moss, microenvironment is vitally important.

Conservation organisations have limited resources, and thus it must be decided which bryophyte species are the most important ones to conserve. But how is this done? What criteria are used to determine the importance of a species? There are many reasons for conserving something e.g. aesthetic value or ecological reasons, but many of these are subjective and depend on the aims and ideals of the conservationists involved (Bisang & Hedenäs 2000). For bryophyte conservation to remain objective, conservation priority needs to be assigned based on scientifically defined criteria.

Distribution and rarity have been the main scientific criteria used to determine conservation priority over the past few decades. Conservation priority is assigned based on whether a taxon is rare or common, and whether it is threatened by human activity or not (Söderström 1995). Those that are rare and threatened are given the highest conservation priority i.e. they are the most important species to conserve.

Rarity can be defined according to Rabinowitz (1981). She defined rarity by three variables – narrow geographical range, high habitat specificity and small population size. These variables can be combined to give eight categories, seven of which can be termed "rare". Using these variables, *Z. leptobolax* can be classified as rare. *Z. leptobolax* has a very small geographical range – it is endemic to a single mountain in South Africa, it has a very specific habitat – thus far it has only been found on one type of tree, and only on old specimens of that tree species, and it has mainly been found in small populations. In addition to its rarity, *Z. leptobolax* is currently threatened by human activity. Plans are in place to remove alien *Quercus* trees from Table Mountain. This would result in the removal of all possible hosts and could lead to the extinction of *Z. leptobolax*. Hence, this moss is both rare and threatened and can be given the highest conservation priority based on the criteria of distribution and rarity.

Over the past few years, molecular based methods for assigning conservation priority have become widely accepted as preferable to other methods. A widely held view is that conservation should try to maximise the amount of genetic information (and as a consequence, biodiversity) that can be preserved. This means that the most genetically distinct taxa should be afforded the highest conservation priority. In some cases, it may be that an endangered species is less genetically distinct than a less threatened one. It would appear that the best conservation strategy in this case would be to conserve the common, but distinct, species, and ensure that maximum genetic variation is preserved (Crozier 1997).

There are several methods for assigning conservation priority using molecular phylogenies, but they are all based on the idea that phylogenetically distinct species are likely to be distinct in other features as well (Hedderson, unpublished manuscript). Another benefit of using molecular phylogenies is that conservation priority can be assigned to populations within a given species. Hence, once a species has been chosen for conservation, a population cladogram can be constructed and this can be used to determine which populations of the species to preserve. In this way, genetic variation and evolutionary potential of a species can be preserved, as some populations may harbour more variation than others (Soltis 1999, Hedderson unpublished).

Zygodon leptobolax is morphologically almost identical to *Z. intermedius*. Without the distinctive synoicous inflorescences, *Z. leptobolax* is virtually indistinguishable from *Zygodon intermedius*. *Z. intermedius* is dioicous, and this character, combined with location of the specimen, is used for identification. If conservation priority were to be assigned on the basis of rarity and distribution alone, *Z. leptobolax* may have been overlooked due to its' similarity to *Z. intermedius*, which is neither rare nor threatened. However, looking at genetic distinctiveness, *Z. leptobolax* should be afforded a high conservation priority and should have conservation efforts directed to its preservation. The phylogenetic tree constructed from *psb*A data shows that *Z. leptobolax* has a high level of genetic distinctiveness when compared to its closest morphological counterpart. In order to preserve the maximum information contained in the genus *Zygodon*, *Z. leptobolax* must be preserved. To lose this species would be to lose a large reserve of potentially unique genes.

There was no within species genetic variation, which indicates that all populations are of equal conservation worth. Populations seemed to be clustered together into three main groups. In addition, most populations were small in size, and only four of them showed evidence of sexual reproduction (sporophytes). This could indicate that there are population processes occurring that are beyond the scope of this study. It is possible that the three clusters form some sort of metapopulation, or that within the three clusters there are metapopulation dynamics at work. The four populations that have reproduced sexually may be "source" or "core" populations, while others may be temporary colonisation sites. This requires further investigation, but if the populations are functioning as a metapopulation it is important not only to preserve all current populations and hosts, but also all potential hosts. This would be essential for the survival of the species.

Z. leptobolax can be afforded a high conservation priority on the basis of rarity, distribution, and genetic distinctiveness. With only a handful of populations remaining, and imminent threat to their habitat, a conservation plan must be devised quickly if we are to preserve this species.

ACKNOWLEDGEMENTS

I would like to thank Professor T.A.J. Hedderson for his help and guidance with the analysis of the molecular data and with the writing up of this study. I also wish to thank Ms. T. Nowells for her assistance with the molecular work, Mr. R. Blanchard and Mr. S. Todd for their generous help with the distribution mapping, and Mr. P. Molenaar for his invaluable assistance with fieldwork.

REFERENCES

Bisang, I., Hedenäs, L., 2000. How do we select bryophyte species for conservation, and how should we conserve them? Lindbergia 25, 62-77.

Crozier, R.H., 1997. Preserving the information content of species: genetic diversity, phylogeny, and conservation worth. Annual Review of Ecology and Systematics 28, 243-268.

Gawel, N.J., Jarret, R.L., 1991. A modified CTAB DNA extraction procedure for Musci and Ipomoea. Plant Molecular Biology Reporter 9, 262-266.

Hamilton, M.B., 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Molecular Ecology 8, 521-523.

Hedderson, T.A., unpublished data. Molecular phylogenetic perspectives on conservation priorities.

Le Maitre, D.C., et. al., 2002. Invasive alien trees and water resources in South Africa: Case studies of the costs and benefits of management. Forest Ecology and Management 160, 143-159.

Magill, R.E., van Rooy, J., 1998. Bryophyta Part 1, Musci. In *Flora of Southern Africa* edited by O.A. Leistner. National Botanical Institute, South Africa.

Rabinowitz, D., 1981. Seven forms of rarity. In *The biological Aspect of Rare Plant Conservation* edited by H.Synge. Wiley, Chichester.

Sim, T.R., 1926. The Bryophyta of South Africa. The Royal Society of South Africa, West Germany.

Söderström, L., 1995. Bryophyte conservation – input from population ecology and metapopulation dynamics. Cryptogamie. Helvetica 18, 17-24.

Soltis, P., Gitzendanner, M.A., 1999. Molecular systematics and the conservation of rare species. Conservation Biology 13, 471-783.